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Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in *Cucumis*

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Summary

Variation at isozyme and random amplified polymorphic DNA (RAPD) loci in eight cucumber and seven melon cultivars, breeding lines, and plant introductions were used to determine the utility of these markers for assessing genetic variation among populations of each species. Although dendrograms derived from cluster analyses using species' variation at marker loci were dissimilar, these disparities were consistent with differences in the pedigrees and/or other information (e.g., morphological) known about each accession and species. Empirical estimations of variances associated with each marker type in the cucumber and melon accessions examined indicate that, per band, lower coefficients of variation can be attained in the estimation of genetic difference when using RAPDs compared to isozymes. The disparity between the marker analyses made may be related to the amount of genome coverage characteristic of a particular marker system in a species and its efficiency in sampling variation in a population.

Introduction

Introduced germplasm continues to be critical to improvement of cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) worldwide. Efforts to collect, preserve and characterize cucumber and melon from their putative centers of origin and diversity have been ongoing for nearly a century. The U.S. Department of Agriculture, National Plant Germplasm System (NPGS), Germplasm Resources Information Network (GRIN) presently lists 1,558 cucumber and 3,253 melon accessions. Because of the modest size of these collections, there is a sense of urgency to add accessions of these crops due to genetic erosion in their centers of origin and diversity (McCreight & Staub, 1993). Future efforts are uncertain because of the need to optimize scarce resources required for germplasm collection, characterization, preservation and utilization. It is, therefore, critical to determine the extent of genetic variation within the existing accessions in order to determine whether additional accessions are warranted, where such germplasm should be collected, and whether collection efforts should be restricted to specific types.

Cucumber and melon belong to the Cucurbitaceae. This family is subdivided into two subfamilies, Zanonioideae and the Cucurbitoideae (Jeffery, 1980; Kirkbride, 1993). The latter subfamily is further partitioned into eight tribes of which the Melothrieae includes the genus *Cucumis*. This genus is partitioned into two subgenera designated as *Cucumis* and *Melo* which contain five cross-sterile species groups (Jeffery, 1980). The subgenus *Cucumis* ($x = n = 7$) comprises three or four Sino-Himalayan species, including *C. sativus* L. The species *C. sativus* houses a number of botanical varieties including *C. sativus* L. var. *sativus* (cultivated cucumber; hereafter referred to as *C. s.* var. *sativus*) and *C. sativus* var. *hardwickii* (Royle) Alef. (a small fruited non-domesticated type; hereafter referred to as *C. s.* var. *hardwickii*) (Schuman et al., 1985). *C.*

s. var. *hardwickii* is being used in cucumber breeding programs as a source of multiple lateral branching and sequential fruiting not characteristic of *C. s.* var. *sativus* (Kupper & Staub, 1988). However, a mutant *C. s.* var. *sativus* type referred to as 'Arkansas Little Leaf' also possesses multiple laterals and appears to have a sequential fruiting habit. Other modern commercial cultivars and breeding lines also have exotic *C. s.* var. *sativus* germplasm in their pedigrees (Peterson, 1975).

Melo ($x = n = 12$), the second subgenus, is divided into two sections (*Melo* and *Aculeatosi*). Section *Melo* contains six series (*Humifructuosi*, *Melo*, *Hirsuti*, *Metuliferi*, *Angurioidei*, and *Myriocarpi*) (Kirkbride, 1993). Series *Melo* houses melon and series *Metuliferi* contains *metuliferus* ("horned cucumber") which is considered a putative bridge species between *C. sativus* and *C. melo* (cultivated melon). Melon consists of a diverse array of types differing in leaf, vine, plant and fruit characters. Wild and cultivated melon in the Series *Melo* is subdivided into two subspecies: *C. melo* L. subsp. *agrestis* (Naud.) Pangalo and *C. melo* L. subsp. *melo*. Subspecies *melo* is further subdivided into six cultivar groups following modern international codes of nomenclature for plants (Bailey & Bailey, 1976; Grebenščikov, 1986; Kirkbride, 1993; Munger & Robinson, 1991; Whitaker & Davis, 1962): 1) Cantalupensis – cantaloupe or muskmelon; 2) Inodorus – winter melons, honeydew, casaba; 3) Flexuosus – snake melon; 4) Conomon – pickling melon; 5) Chito and Dudaim – mango melon, vine peach and other similar names for the former; pomegranate melon, Queen Anne's Pocket melon for the latter, and, 6) Momordica – 'phut' or snap melon.

Isozymes, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers have been used for diversity analysis, the construction of genetic maps, and marker-aided selection (Staub et. al., 1996a). The inheritance and linkage relationships of 21 isozyme loci in cucumber has been determined (Knerr & Staub, 1992; Meglic & Staub, 1996).

Isozymes and RFLPs have been used to assess the genetic diversity of *C. sativus* (Knerr et al., 1989; Dijkhuizen et al., 1996) and to determine species relationships (Staub et al., 1992a; Perl-Treves & Galun, 1986a; Perl-Treves et al., 1986b). In addition, a 58-point map has been constructed in *C. sativus* using isozyme, RAPD, RFLP, morphological and disease resistance loci (Kennard et al., 1994). Nevertheless, the comparative efficiency of RAPDs and isozymes for determining intraspecific relationships in *C. sativus* has not been reported.

Neuhausen (1992) used RFLP markers for melon cultivar discrimination. DNA sequence variation examined by polymerase chain reaction (PCR) amplification and direct sequencing has revealed polymorphism among melon cultivars (Shattuck-Eidens et al., 1990). Repetitive sequence variation (satellite DNA) has been used for taxonomic classification of melon (Zentgraf et al., 1992; Torres-Ruiz & Hemleben, 1991). Isozyme analysis has been applied to cultivar identification and breeding (Chen et al., 1990; Sujatha et al., 1991; Sujatha & Seshadri, 1991).

The potential utility of RAPD for genetic or diversity studies in *Cucumis* has not been documented. However, the genetic diversity of the U.S. NPGS melon collection was assessed using 16 isozyme loci (Meglic et al., 1994). Two of these 16 loci segregate independently and 14 have been assigned to three linkage groups. Esquinas-Alcazar (1977) grouped melons from 11 countries of origin into four groups using isozyme loci. The first group included germplasm from Israel, India and Spain, and the second group included accessions from Afghanistan, China, France, India and Mexico. The third group included accessions from China, Korea and Japan, while accessions from Russia and U.S.A. comprised a fourth group. From ancient tradition of melon culture, Esquinas-Alcazar (1977) reasoned that India might be the founder of the groups one and two, and China might be the founder of groups three and four. His data also indicated that U.S.A. cultivars show biochemical affinities with Spanish melons.

Isozymes have been used for the assessment of genetic diversity in cucumber and melon (Esquinas-Alcazar, 1977; Knerr et al., 1989; Meglic et al., 1994; Meglic & Staub, 1996). Isozyme analysis allows for grouping of countries by geographic proximity (Meglic & Staub, 1996). However, the paucity of isozyme loci permits only limited discrimination among cucumber cultivars (Staub & Meglic, 1993). Despite the sensitivity of RAPDs to PCR conditions and reaction reagents (Staub et al., 1996b), studies in other crops indicate the potential of RAPD markers for assessment of genetic diversity (Yu & Pauls, 1993; Beebe et al., 1995). Reliable RAPD markers in conjunction with isozyme and morphological markers will allow for more rigorous assessments of germplasm management strategies and assist in the planning of *Cucumis* collection expeditions.

Here we show the utility of RAPDs for examining intraspecific relationships among domesticated (*C. s.*

Table 1. Description of cucumber (*Cucumis sativus* L.) and melon (*C. melo* L.) varieties, line and plant introductions used in intraspecific comparisons by isozyme and random amplified polymorphic DNA marker loci analysis.

Variety, line or plant introduction number	Species	Chromosome number (2n)	Plant habit[z]	Morphologic characteristics of fruit[y]
Cucumber				
WI 2757	*C. sativus* var. *sativus*	14	*De*	Smooth skin, spines white and fine, L/D = ∼ 2.6
G421	*C. sativus* var. *sativus*	14	*de*	Warty skin, spines white and coarse, L/D = ∼ 2.8
GY-14	*C. sativus* var. *sativus*	14	*De*	Warty skin, spines white and coarse, L/D = ∼ 2.9
H-19	*C. sativus* var. *sativus*	14	*De*	Warty skin, spines white and coarse, L/D = ∼ 3.2
PI 183967	*C. sativus* var. *hardwickii*	14	*De*	Warty skin, spines black and coarse, L/D = ∼ 2.1
PI 269480	*C. sativus* var. *sativus*	14	*De*	Warty skin, spines white and coarse, L/D = > 5.0
PI 458845	*C. sativus* var. *sativus*	14	*De*	Warty skin, spines white and coarse, L/D = > 5.0
PI 432860	*C. sativus* var. *sativus*	14	*De*	Warty skin, spines white and coarse, L/D = > 5.0
Melon				
Doublon	*C. melo* subsp. *melo* Cantalupensis Group	24	–	Globular; traces of net; blue-green skin with dark green vein tracts; orange flesh; sweet; ∼ 1.0 kg
Freeman Cucumber	*C. melo* subsp. *melo* Conomon Group	24	–	Globular; smooth, green skin; white flesh; ∼ 0.5 kg
Green Flesh Honeydew	*C. melo* subsp. *melo* Inodorus Group	24	–	Globular; smooth, ivory skin; green flesh; sweet; 1.1–2.2 kg
Juana Canari	*C. melo* subsp. *melo* Inodorus Group	24	–	Globular; smooth, yellow skin; white flesh; sweet; 1.1–2.2 kg
Snakemelon	*C. melo* subsp. *melo* Flexuosus Group	24	–	Elongate; smooth, ivory or dark green skin, may be striped; green or white flesh; 1–10 kg, 0.2–2.0 m
Top Mark	*C. melo* subsp. *melo* Cantalupensis Group	24	–	Globular; thick, deep, dense net; shallow, net-covered vein tracts; tan skin; orange flesh; abscises at maturity; sweet; 1.0–2.2 kg
PI 124111	*C. melo* subsp. *melo* Momordica Group	24	–	Flattened; smooth, dark green skin; white flesh; dehisent at maturity; < 0.5 kg
Horned Cucumber	*Cucumis metuliferus*	24	–	Oblate; warts; pale green skin, pale green, mealy flesh; < 0.5 kg

[z] *De* = indeterminate, vining habit, *de* = indeterminate habit where apical whorls terminate in a cluster of flowers.
[y] L/D = length : diameter ratio of mature fruit.

var. *sativus*) and wild (*C. s.* var. *hardwickii*) cucumbers, and among five of the six groups of cultivated melons. RAPD and isozyme marker variation is related to previous taxonomic classification and available pedigree information of cucumbers and melons included herein. A variance sampling procedure (bootstrap) was employed to compare the efficiency of RAPD and isozyme markers for characterizing intraspecific relationships.

Materials and methods

Germplasm

Cucumber. Cucumber lines and plant introductions (PI) used represented the morphological range in *C. s.* var. *sativus* (Table 1). The inbred processing type cucumber lines GY-14a and G421 were received from R. L. Lower, University of Wisconsin-Madison. GY-14a is a standard indeterminate monoecious line possessing normal-size leaves. G421 is a determinate gynoecious line with normal-sized leaves. The indeterminate, multiple branching, monoecious inbred line "Arkansas Little Leaf" (H-19) has little leaves, and was developed at and received from the University of Arkansas, Fayetteville, AR. The leaf area of first fully expanded leaf of H-19 is 30 to 40, while of standard leaf types is 80 to 100 cm^2 (Staub et al., 1992b). The multiple disease-resistant inbred line WI 2757 (USDA, ARS) is indeterminate, gynoecious, smooth-skinned, fine-spined and was developed from several exotic sources of germplasm (Peterson et al., 1982). The *C. s.* var. *sativus* PI 269480 (Pakistan), PI 432860 (China) and PI 458845 (Russia; former Soviet Union) were obtained from NPGS. These indeterminate, monoecious, normal-leafed PIs were found to exhibit isozyme and RFLP variation different from North American-adapted cultivars and many PIs in the U.S. collection (Knerr et al., 1989; Dijkhuizen et al., 1996; Kennard et al., 1994). The *C. s.* var. *hardwickii* PI 183967 (India) differs from var. *sativus* in morphology (Kupper & Staub, 1988), isozyme (Knerr et. al., 1989) and RFLP (Kennard et al., 1994; Dijkhuizen et al., 1996)

variation. It is an indeterminate, monoecious, multiple branching plant type with little leaves. The *C. metuliferus* C79298 (GBNR 1985) was received from CPRO Wageningen, The Netherlands, and was used as an outgroup (distant species) for comparison.

Melon. Melon cultivars, breeding lines and PIs were chosen from five of the six cultivated Groups of *C. melo* subsp. *melo* (Table 1). 'Top Mark' and 'Doublon' are representative of the Cantalupensis Group. 'Top Mark' is the current type for the orange-fleshed, western U.S. shipping-type melon and was developed in the 1960s by Dessert Seed Company, El Centro, CA (seeds received from Harris Moran Seed Company, CA). 'Doublon' is a French cultivar released by Institut National de la Recherche Agronomique, France (USDA seed increase) is resistant to race 1 of *Fusarium oxysporum* f. sp. *melonis* (Risser, 1973). 'Green Flesh Honeydew' (Honeydew; seeds received from Northrup-King Seed Company, CA) and 'Juane Canari' (Canari; seeds received from Sluis and Groot Seed Company, CA) are distinctly different representatives of the Inodorus Group. Honeydew was introduced into the U.S.A. about 1890 (Whitaker & Davis, 1962). 'Canari' is similar to 'Golden Beauty Casaba' which was introduced into the U.S.A. about 1878 (Whitaker & Davis, 1962). The Conomon Group is represented by 'Freeman Cucumber' (seeds obtained from H.M. Munger, Cornell University, NY) which is resistant to cucumber mosaic virus (Enize, 1943), and the Flexuosus Group is represented by Snakemelon from Beni Suef in upper Egypt (seeds obtained from A. Ibrahim, Riyadh, Saudia Arabia). The Momordica Group is represented by PI 124111 collected in Calcutta, India in 1937 (seeds obtained from NPGS), which is known its for resistance to powdery mildew incited by *Sphaerotheca fuliginea* and *Erisyphe cichoracearum* (Harwood & Markarian, 1968) and downy mildew caused by *Pseudoperonospora cubensis* (Thomas et al., 1988). A bonafide sample of the Chito Group was not readily available.

Sample preparation and electrophoresis

Isozymes. Melon and cucumber cotyledons were harvested from 7-day-old seedlings which were germinated and grown in vermiculite for electrophoretic evaluation. Samples were bulked for analysis such that approximately 0.01 g of cotyledonary tissue from each seedling was ground in 0.1 ml of a buffer solution containing 0.67 g/l Tris base and 7.02 g/l Tris-HCl at pH 7.1. Ground samples were held at 5°C ($<$ 2 hrs) before horizontal starch gel electrophoresis according to Knerr & Staub (1992). Modified staining solutions of Allendorf et al. (1977), Brewer (1970), and Shaw & Prasad (1970) were used to visualize reproducible isozyme banding patterns in 17 enzyme systems (Table 2).

Gels consisted of either 42 g or 56 g of a 1:1:1 mixture of hydrolyzed potato starch (Sigma Co., St. Louis, MO, U.S.A.), Connaught starch (Connaught Laboratories, Willowdale, Ontario, Canada), and Starch Art hydrolyzed potato starch (Starch Art, Smithsville, TX, U.S.A.) dissolved in either 300 ml or 400 ml of buffer, respectively. Gel and electrode buffers described by Allendorf et al. (1977; A), Clayton & Tretiak (1972; C), Markert & Faulhaber (1965; M), Ridgway et al. (1970; R), and Selander et al. (1971; S-4) were used. These will be referred to as A (pH 7.1 gel, 7.0 electrode), C (pH 6.1 gel and electrode), R (pH 8.5 gel, 8.1 electrode), S-4 (pH 6.7 gel, 6.3 electrode), and M (pH 8.7 gel and electrode). To visualize isozymic variation in malate dehydrogenase (MDH), S-4 gel buffer was adjusted to pH 6.2 and electrode buffer to pH 5.8 (Knerr et al., 1995).

Isozyme variation observed at 21 and 20 mapped loci was recorded for analysis in cucumber and melon, respectively (Meglic & Staub, 1996; Meglic et al., 1994). Genetic nomenclature used to describe allozymic variation followed a form (Knerr & Staub, 1992) previously described by Richmond (1972).

RAPDs. Standard CTAB phenol:chloroform extraction procedures were modified to optimize DNA quality and quantity in a mini-prep format (Staub et. al., 1996b). About 0.1 to 0.3 g of frozen, pulverized tissue was subjected to various phenol-chloroform-isoamyl alcohol extraction procedures. The resulting solution was stored at -20°C.

PCR amplifications were performed in 15 ml reaction volumes containing 50 mM KCl, 10 mM Tris (pH 9.0), 0.1% Triton X-100, 3.0 mM $mgCl_2$, 0.2 mM of each of the dNTPs, 0.33 mM 10 mer primer [Operon Technologies, Alameda, CA; University of British Columbia (BC), Vancouver, BC, Canada], 15 ng of genomic DNA, and 1 Unit of Taq DNA polymerase (Promega, Madison, WI). DNA was quantified by fluorometry using a TKO 100 mini-fluorometer (Hoefer Scientific Supplies, San Francisco, CA). PCR was performed in a Perkin Elmer Cetus 9600 thermocycler (Norwalk, CT) programmed for: 1 cycle @ 94°C/4 min.; 3 cycles @ 94°C/15 sec., 35°C/15 sec., 72°C/75

Table 2. Enzymes assayed, buffer systems used and loci examined in an evaluation of cucumber (*Cucumis sativus* L.) and melon (*C. melo*) genotypes.

Enzyme	Abbreviation	E.C.[z] designation	Buffer system[y]	Cucumber loci observed[w]	Melon loci observed[v]
Aconitase	AC	4.2.1.3	S-4	–	1[4]
Acid phosphatase	ACP	3.1.3.2	C	–	2
Adenylate kinase	AK	2.7.4.3	S-4	2	1
Fructose diphosphatase	FDP	3.1.3.11	A	2	2
Glucosephosphate isomerase	GPI	5.3.1.9	R	1	1
Glutathione reductase	GR	1.6.4.2	S-4	1	–
Glycerate dehydrogenase	G2DH	1.1.1.29	R	1	–
Isocitrate dehydrogenase	IDH	1.1.1.42	S-4	1	1
Malate dehydrogenase	MDH	1.1.1.37	S-4	3	4
Manosephosphate isomerase	MPI	5.3.1.8	S-4	2	2
Peptidase with glycyl-leucine	PEP-GL	3.4.13.11	A	2	1
Peptidase with leucyl-alanine	PEP-LA	3.4.13.11	M/S-4[x]	1	1
Peptidase with phenylalanyl-proline	PEP-PAP	3.4.13.11	S-4	1	–
Peroxidase	PER	1.11.1.7	A	1	1
Phosphoglucomutase	PGM	5.4.2.2	R	1	1
6-phosphogluconate dehydrogenase	PGD	1.1.1.43	S-4	2	1
Shikimate dehydrogenase	SKDH	1.1.1.25	S-4	1	1

[z]Enzyme commission number.
[y]Buffers of Clayton and Tretiak (1972), Ridgway et al. (1970), and Selander et al. (1971), Markert and Faulhaber (1965) designated as C, R, and S-4 or M, respectively. Buffer S-4 is a modification of the original Selander et al. (1971) buffer where the pH is 6.7 gel, and 6.3 electrode. MDH analyzed using S-4 as described by Knerr et al. (1995).
[x]Buffer system M used for cucumber and S-4 used for melon.
[w]Loci designated by previous examination (Knerr and Staub, 1992; Meglic and Staub, 1996) or during this survey using standard criteria and nomenclature (Richmond, 1972).
[v]Loci designated by previous examination (Meglic et al., 1994) where loci were assigned using nomenclature of Richmond (1972).

sec. (ramp time 59 sec.); 40 cycles @ 94°C/15 sec., 40°C/15 sec., 72°C/75 sec. (ramp time 59 seconds); 1 cycle @ 72°C/7 min; and then 4°C soak.

All RAPD reaction products were electrophoresed in 20 × 25 cm, 1.6% agarose gels with 0.5 μg/ml of ethidium bromide in 1X TAE buffer. Gels were run for 3 h at 93 volts in Gibco/BRL H4 gel apparatus (Bethesda, MD), illuminated by UV light and photographed with an Eagle Eye still video system (Stratagene, LaJolla, CA). Banding variation (presence or absence) was recorded in 11 RAPD primers that allowed for the examination of 43 loci (OPF4_1-3, OPH5_1-3, OPAE6_1-4, OPAK16_1-5, OPAL5_1-7, OPAO8_1-2, OPAW14_1-4, BC226_1-4, BC388_1-4; BC526_1-4, and BC551_1-3) which had previously been found to segregate in a predictable 3:1 Mendelian ratio in cucumber (Kennard et. al., 1994) and melon (unpublished data).

Analytical procedures

Similarity comparisons. Cluster analysis using the PROC CLUSTER subroutine of SAA/STAT was utilized to depict relationships among *C. sativus* and *C. melo* inbred lines, cultivars, and PI's (SAS, 1992). Analyses were performed using allelic frequency and genetic difference matrices. Allele frequency was assumed to be $p = 0.5$ and $q = 0.5$ in bulked samples of each accession. In linkage studies using isozymes used in this study, allelic frequencies were not significantly ($P = 0.05$) different from $p = 0.5$ and $q = 0.5$ (Knerr and Staub, 1992, Meglic and Staub, 1996). In addition, a Chi-square analysis of a random sample of 10 heterozygous PIs and breeding lines indicated that allelic frequencies at 10 loci were not significantly different ($P = 0.05$) than $p = q = 0.5 \pm 0.04$ (unpublished data). The RAPD loci used were chosen for their reproducibility (Staub et al., 1996b), segregation,

and relative independence from each other based on their map position (Kennard et al., 1994, unpublished data). Thus, estimates of allelic frequencies were calculated according to the protocol of Widrlechner et al. (1992), and genetic similarity coefficients were calculated using formulae of Nei & Li (1979).

Data (allelic frequency and derived genetic distance matrices) from each species were analyzed separately. To make the data sets more amenable to interpretation (Pearce, 1969), complete linkage cluster analysis (Sokal & Michener, 1958; Sorensen, 1948) was used to group entries. In complete linkage analysis, the distance between two clusters is the maximum distance (MDC) between an observation in one cluster and an observation in the other cluster (SAS, 1992). Clusters with small variances tend to be joined and the clusters which are produced are biased towards having equal variances.

This clustering procedure was choosen because its distance estimating and combinational formulae are coherent and make simplistic mathematical assumptions (Sokal & Michener, 1958; Sorensen, 1948). Clusters were merged sequentially based on their Euclidean distance using an algorithm which initially used each region as a cluster. MDC is therefore the average Euclidean distance between two clusters derived from empirical measurement in multidimensional space. Entries with similar isozyme or RAPD phenotypes were placed in close proximity on a resulting dendrogram.

Marker efficiency comparisons. In order to determine the efficiency of each marker type per unit information (band), the variance within each marker data set (2 isozyme and 2 RAPD) was empirically estimated using a bootstrap sampling procedure (Efron & Tibshirani, 1986). A subset of a given number of N polymorphic bands were generated. The N bands were selected at random from the entire set of possible bands for each data set. Sampling was done with replacement, thus allowing for the probability of 1/N of bands per data set to be selected at any one time from the data set. The band usage frequency was continuously monitored to detect any bias in the use of bands resulting from a total of 100 subsamples (bootstraps). The coefficient of variation (CV) was used to determine the magnitude of the variance because there is a linear relationship between genetic variances and means of distance estimates (Tivang et al., 1994). Comparisons among marker types can be made since differences in the variances of each data set can be normalized.

Results and discussion

Genetic relationships

Our intent was not to define the evolutionary relationships among the *C. sativus* or *C. melo* subsp. *melo* groups, but rather to determine whether the results obtained from the isozyme and RAPD markers were similar to each other and to other taxonomic classification schemes. *Cucumis* intraspecies relationships resulting from cluster analyses derived allelic frequency and genetic distance matrices were similar (data not presented), and thus only cluster analyses using allelic frequency data are presented. In each comparison (Figures 1 and 2), the *C. metuliferus* accession examined was distinctly different from the *C. sativus* and *C. melo* entries. Although Norton & Granberry (1980) reported successfully crossing *metuliferus* with *melo*, this has yet to be confirmed or duplicated by other investigators. Our results lend support to the genetically distant relationship between *C. metuliferus* and *C. sativus* and between *C. metuliferus* and *C. melo*.

Cucumber. Genetic distances (GD) among PI 269480, PI 432860, PI 458845, PI 183967, and GY-14a have been assessed by 10 isozyme and 104 RFLP loci (Dijkhuizen et al., 1996). Variation detected at RFLP and isozyme loci produced similar results. The *C. s.* var. *hardwickii* PI 183967 was distinct from all other entries examined. WI 2757 and GY-14a were found to be more similar to each other than to PI 269480, PI 432860, or PI 458845 which were themselves individually distinct. The genetic distance between WI 2757 and GY-14a was relatively large.

In our study, *C. s.* var. *hardwickii* formed a cluster distinct from the var. *sativus* entries, except for WI 2757 (Figure 1, panel A) and H-19 (Figure 1 B). These results are consistent with morphological observations made when initially attempting to transfer the sequential fruit setting ability ($>$ 100 fruit per plant for certain accessions) and multiple branching habit from *C. s.* var. *hardwickii* to var. *sativus* American processing types (on average $\sim$1.25 fruit per plant per harvest; Miller & Hughes, 1969) (Kupper & Staub, 1988). These traits rapidly approached that of the var. *sativus* parent with selection for fruit and growth characters necessary for commercial acceptance in the U.S. (Fredrick & Staub, 1989).

The *C. s.* var. *sativus* line H-19 has morphological characteristics in common with *C. s.* var. *hardwickii*, and the pedigree of WI 2757 is complex, having many

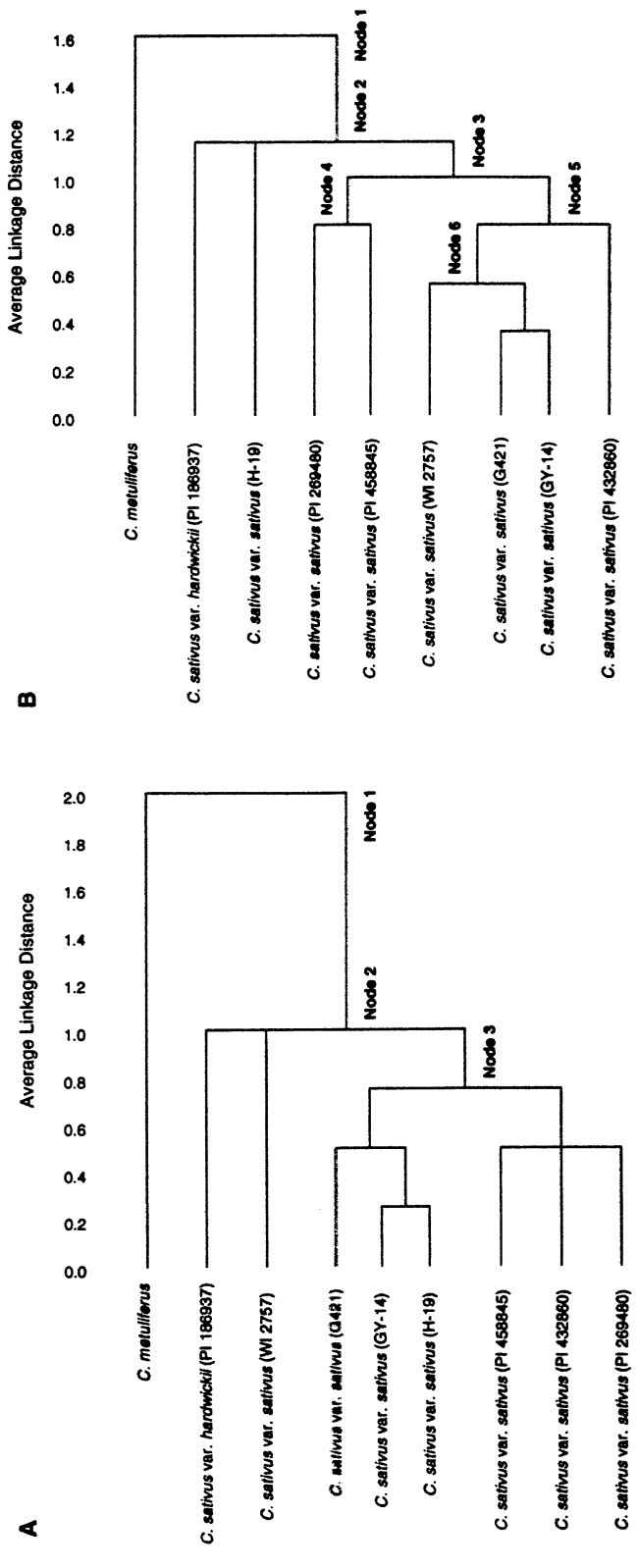

Figure 1. Complete linkage cluster analysis (average linkage distance) of eight *C. sativus* cultigens grouped using 21 enzyme loci (panel A) and 43 random amplified polymorphic DNA loci (panel B).

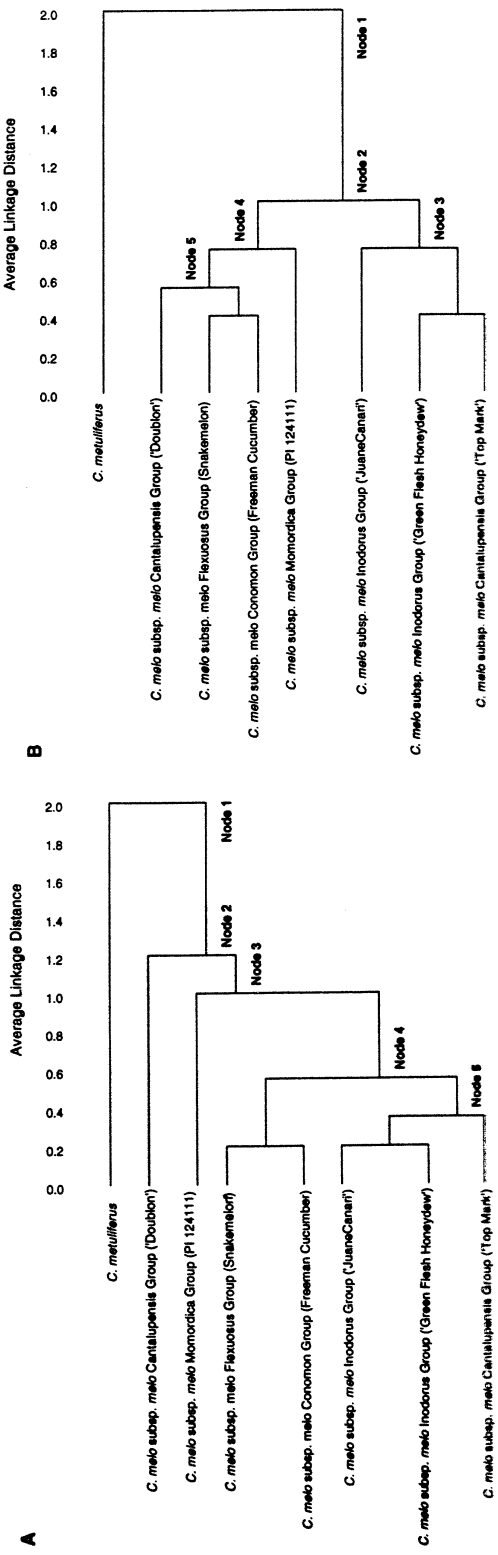

Figure 2. Complete linkage cluster analysis (average linkage distance) of seven *C. melo* cultigens grouped using 20 enzyme loci (panel A) and 43 random amplified polymorphic DNA loci (panel B).

exotic (North American unadapted) var. *sativus* accessions in its parentage (Peterson et al., 1982). Isozyme variation in WI 2757 resulted in its partitioning to a node (node 2) distinct from the other *C. s.* var. *sativus* entries analyzed (Figure 1, panel A). RAPD analysis resulted in the placement of WI 2757 in a cluster with GY-14a (Figure 1, panel B; node 6). Nevertheless, G421 was more similar to GY-14a (GD = 0.87) than WI 2757 (GD = 0.76) regardless of the marker type used. WI 2757 has a complicated pedigree consisting of many cultivars (e.g., 'Expo'; The Netherlands) and PIs [e.g., PI 220860 (Korea; source of gynoecy); PI 197087 (India; anthracnose resistance source), PI 212233 (Japan; powdery mildew resistance source)] unadapted to North American growing conditions, and is morphologically distinct (e.g., thin-skinned, non-warty, fine-spined, etc.; Peterson et. al., 1982). These results agree with previous observations using isozyme and RFLP analyses (Dijkhuizen et al., 1996).

Allozymic variation in H-19 resulted in its placement on a node common with G421 and GY-14a (Figure 1, panel A; node 3). In contrast, H-19 possessed RAPD variation that was distinct from all other *C. s.* var. *sativus* entries (Figure 1, panel B; node 2). H-19 originated as a spontaneous mutant from matings between PI 197087 and 'Model' (personal communication, Ted Morelock, University of Arkansas, Fayetteville, 1996). It is distinct in its plant habit sharing many traits in common with *C. s.* var. *hardwickii* (e.g., multiple lateral branching, sequential fruiting, small leaves, delayed flowering). The inability to distinguish H-19 from G421 and GY-14a may be due to the paucity of isozyme loci (21) examined when compared to RAPD loci (43) in this study.

PI 458845, PI 432860 and PI 269480 have been shown to be distinct from all other accessions in the U.S. collection (including GY-14a) in isozyme and RFLP variation (Dijkhuizen et al., 1996; Knerr et. al., 1989). These PIs formed a single cluster based on isozyme variation in this study distinct from GY-14a (Figure 1, panel A; node 3). Analysis of RAPD data resulted in the grouping of PI 269480 and PI 458845 into one cluster and WI 2757, G241, GY-14a and PI 432860 into another (Figure 1, panel B; nodes 4 & 5). It appears that PI 432860 is more similar to GY-14a (GD = 0.82) than PI 269480 or PI 458845 (GD = 0.63). Nevertheless, in each analysis, these PIs were distinct from GY-14a, thus recapitulating previous studies (Dijkhuizen et al., 1996).

Melon. Isozymic profiles resulted in the placement of 'Doublon' to node 2 which differentiated it from all other melons (Figure 2, panel A). While allozymic variation of PI 124111 was distinct from the remaining melons (node 3), variation in Snakemelon and Freeman Cucumber was similar (node 4). 'Juane Canari' and 'Green Flesh Honeydew' formed a cluster which was distinct from 'Top Mark' (node 5).

RAPD analyses resulted in the placement of 'Doublon' in a cluster with Snakemelon and Freeman Cucumber (Figure 2, panel B, node 5). This group was more similar to PI 124111 than the remaining melon types (node 4). The RAPD variation observed among 'Juane Canari', 'Green Flesh Honeydew', and 'Top Mark' was similar, and thus resulted in their placement in a unique cluster (node 3). This result and the genetic similarities observed between Snakemelon and Freeman Cucumber were similar to that of isozyme analysis.

The melon groups are morphologically distinct and can often be grouped based on character differences at one locus. Such single-locus differences may not adequately represent the genetic diversity present in a species. The separation of 'Doublon' and 'Top Mark' by molecular marker analysis was predicted based on their morphology. Although similar for consistent presence of fruit surface netting and flesh color, 'Doublon' and 'Top Mark' (Cantalupensis Group) differ greatly for a number of characteristics. The classification scheme of Naudin (1859) would have placed 'Doublon' in *C. melo* var. *cantalupensis* and 'Top Mark' in *C. melo* var. *reticulatus* (Whitaker & Davis, 1962; Munger & Robinson, 1991). 'Doublon' is monoecious and its fruit with soft flesh do not abscise at maturity. Although its fruit are lobed and sparsely netted (<50%), the prominent green vein tracts of mature fruit are not covered with net. The blue-green skin color of 'Doublon' becomes creamy-yellow during ripening. In contrast, the fruit of 'Top Mark' are not lobed, but are densely netted (>75%). Its vein tracts are not prominent, and are covered with a netted surface. 'Top Mark' is andromonoecious and its firm-fleshed fruit abscise at harvest. Like 'Doublon', the dark green skin color of 'Top Mark' becomes creamy-yellow during ripening, and both have orange-colored mesocarp.

Disparities were observed among different types of genetic information (i.e., morphological, isozyme and RAPD). These disparities might have been expected since some of morphological characteristics inherent to the melon types studied could not be directly related to marker variation or their underlying DNA sequences. For instance, both molecular analyses found similari-

ties among 'Juane Canari', 'Green Flesh Honeydew', and 'Top Mark' (Figure 2, panels A & B). These analyses are consistent with the data of Esquinas-Alcazar (1977). 'Juane Canari' and 'Green Flesh Honeydew' (Inodorus Group; 'winter melons') differ greatly from 'Doublon' and 'Top Mark' (Cantalupensis Group) for many horticultural characteristics. The fruit of 'Green Flesh Honeydew' and 'Juane Canari' do not abscise at maturity. 'Green Flesh Honeydew' and 'Juane Canari' differ from each other in fruit shape, skin color, surface texture and flesh color. The exterior surface of 'Green Flesh Honeydew's' round fruit are smooth and possess an ivory-green colored epidermis and mesocarp. In contrast, the epidermis of the ellipsoid fruit of 'Juane Canari' is wrinkled and the skin color is gold-yellow and green. Although its mesocarp is ivory-colored, a slight salmon tint can occur near the endocarp (i.e., seed cavity).

In this study, isozyme analysis indicated that Snakemelon (Flexuosus Group) and Freeman Cucumber (Conomon Group) had greater biochemical affinities to 'Top Mark' (Cantalupensis Group), 'Green Flesh Honeydew' (Inodorus Group) and 'Juane Canari' (Inodorus Group) than to 'Doublon' (Cantalupensis Group) or PI 124111 (Momordica Group) (Figure 2, panel A). In contrast, RAPD variation suggests that Snakemelon and Freeman Cucumber are more similar to 'Doublon' and PI 124111 than to 'Top Mark', 'Green Flesh Honeydew', and 'Juane Canari' (Figure 2, panel B). The lack of agreement between marker types in melon may be due to the difference in the number of isozyme and RAPD loci examined. Melons are cross-compatible, and thus the degree of genetic similarity among the melon types examined depends on the extent of introgression which has occurred among them. Whitaker & Bohn (1954) suggested that Conomon Group (Freeman Cucumber) may have originated in China and Korea. Flexuosus Group (Snakemelon) is common throughout India and China. PI 124111 (Mormordica Group) is of Indian origin. Individual vine and fruit and characteristics of these three melons can be found in 'Doublon', 'Top Mark', 'Honeydew' and 'Juane Canari'. Further genetic analyses of these groups is required to determine the extent of genetic introgression.

Marker efficacy

Sample variance occurs during the estimation of genetic relationships when a random subset of variables (e.g., isozymes and RAPDs) does not equal the value

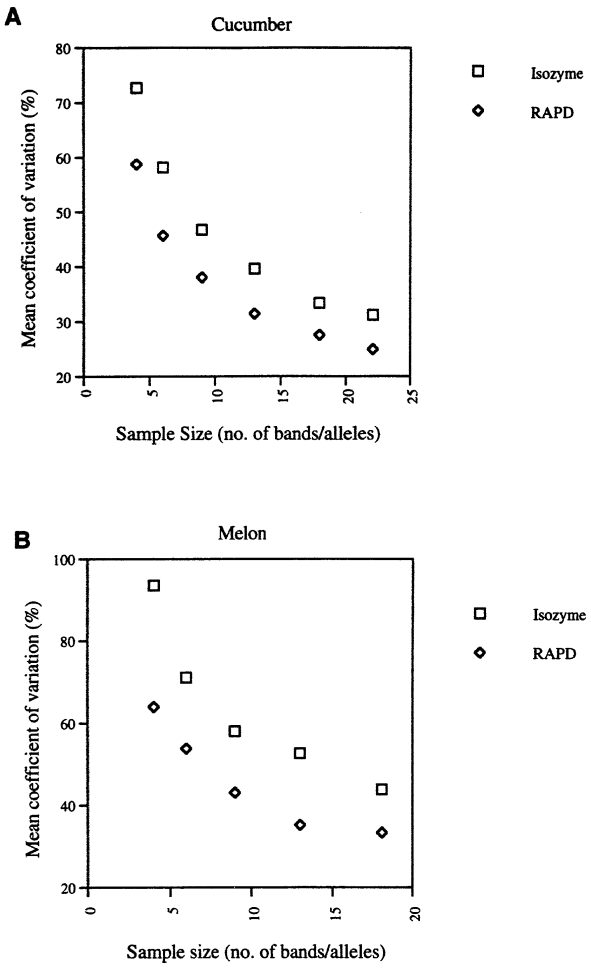

Figure 3. Sample variance of genetic distance estimation for *C. sativus* (panel A) and *C. melo* (panel B) as depicted as the relationship between the mean coefficient of variation (%) and the sample size [number of RAPD and isozyme bands (alleles)] derived from a bootstrap procedure.

that would be obtained from sampling the entire population. As the number of random measurements (e.g., marker bands) increases, the distribution of values taken from a subset of the population becomes more uniform. As the variance is reduced by sampling larger population sizes, estimations of genetic relationships become more definitive since over- or undersampling certain regions of the genome is decreased (Tivang et al., 1994).

Examining the relationship between the CV and the sample size (number of bands) used can provide information concerning the variation associated with estimations of genetic differences (Tivang et al., 1994). As more bands are utilized more information is

gained (i.e., lower CVs are attained). Empirical estimations of variances associated with each marker type in this study indicate that, per band, lower CVs can be attained when using RAPDs compared to isozymes (Figure 3). The mean CV when sampling 21 isozyme loci in cucumber was ∼30% compare to ∼25% using 21 RAPD bands. Likewise, sampling 20 isozyme loci in melon resulted in a CV of ∼43% compared to 33% for RAPDs. These data suggest that RAPD loci may allow for a higher level of discrimination when compared to the isozyme loci used.

The potential utility of isozyme and RAPD markers for germplasm management can be evaluated where taxonomic/pedigree relationships are known and comparative information can be obtained for analysis (e.g., from studies utilizing common accessions). There were disparities observed in our study when cucumber and melon entries were grouped based on marker type (Figures 1 and 2). A knowledge of the inheritance and map location of the markers used in any diversity assessment can enhance its value. The breadth of variation detected in populations may be constrained by the variation present between mapping parents used to construct a particular map, and the type (i.e., codominant vs. dominant), genomic distribution and total genome length characterized by the markers used (Kennard et al., 1994; Dijkhuizen et al., 1996). The use of an array of mapped and independent markers may increase discrimination during diversity assessment if they are well distributed (i.e., minimal clustering) and cover a substantial portion of the genome. In this study, a greater number of RAPD loci were used to depict intraspecific relationships than isozyme loci. Although the genome length and distribution of the RAPD markers used to examine variation among the melon entries used in our study is not known, genome coverage using isozyme markers in our study was 98 cm with an interval distance between markers of 8.9 cm (Meglic et al., 1994). The estimated genome length of cucumber is between 750 and 1000 cm (Staub & Meglic, 1993). The map distance covered by the isozyme and RAPD loci used in cucumber was ∼270 cm and ∼630 cm, respectively (Meglic & Staub, 1996; Kennard et al., 1994; unpublished data). The interval distance between isozyme and RAPD markers is 14.8 and 7.6 cm, respectively. These maps and the RFLP maps available for cucumber (Kennard et al., 1994) have not been integrated, and thus the position and linkage relationships between the loci employed in this study are not known.

Isozyme and RFLP loci were equally effective in discriminating a diverse array of 16 cucumber entries (Dijkhuizen et al., 1996). Several entries examined by Dijkhuizen et al. (1996) were used in our study. Grouping of these accessions by isozymic profiles agreed with that of Dijkhuizen et al. (1996) and available pedigree information. Grouping of accessions by RAPDs might have been predicted to provide a different description of intraspecific relationships given the greater number of RAPD markers employed, the sampling efficiency of RAPDs, and greater length of genome coverage provided by RAPDs when compare to the isozymes employed in this study. Analysis of RAPD variation suggests that the *C. s.* var. *sativus* cultivar H-19 and the *C. s.* var. *hardwickii* accession PI 183967 appear to share genetic similarities. H-19 and PI 183967 also share similarities in their flowering response and plant habit. RAPD analysis also identified genetic similarities between PI 432860 and GY-14a not previously described by Dijkhuizen et al. (1996). These accessions do not share many common morphological similarities. Isozyme and RFLP analysis grouped 'Juane Canari', 'Green Flesh Honeydew', and 'Top Mark' melons into one cluster. Likewise, Snakemelon and Freeman Cucumber were consistantly grouped together. These groupings are consistent with known taxonomic information. Analysis of RAPD suggested that a closer genetic relationship exists among 'Doublon', and Snakemelon and Freeman Cucumber than that ascribed by isozyme analysis. The difference in groupings observed between RAPD and isozyme analysis may be due to the greater number of RAPDs used in the analysis.

In cucumber, isozymes have been used to characterize relationships among accessions in the U.S. NPGS (Knerr et al., 1989; Meglic et al., 1996) and cultivars (Staub & Meglic, 1993). RAPD markers have been used to identify genetic relationships among accessions in various collections to aid in the germplasm management of plant species (Nienhuis et al., 1995; Ren et al., 1995). Marker utility in germplasm management and improvement will ultimately be determined by cost/unit information (Staub et al., 1996a). In this study, RAPD loci were found more efficient than isozyme loci for detecting variation on a per band basis. It is unclear, however, which marker type is most cost effective and their use will depend upon specific research objectives. Laboratory costs associated with germplasm assessment and marker-assisted selection applications are decreasing, and more effective and efficient molecular markers are being developed. For instance, fluorescence techniques are being used to increase the reliability of PCR reactions for large-scale

screening of germplasm (Gu et al., 1995) and improved isozyme procedures such as cellulose acetate (Goodwin et al., 1995) are being used to improve efficiency of isozyme analysis.

Marker technology will be used increasingly for the germplasm management and improvement of cucumber and melon as cost effective marker types are identified and their genetic bases understood. One approach to germplasm management which might be employed to better clarify the genetic structure of the U.S. NPGS cucumber and melon collections is the strategic utilization of both isozymes and RAPDs. Given the low cost, efficiency and repeatability of isozyme analysis, this co-dominant marker system might be used initially to survey a *Cucumis* collection whose genetic structure is not known. RAPD analysis might then be employed to investigate genetic relationships between observed groupings and among individuals in groupings identified by isozyme analysis. Tandem use of marker systems such as these will become increasely attractive as the genome structure of these species is more clearly defined.

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